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Analysis of Apparent Catalytic Parameters of Multiple Molecular Forms of Human Plasma Butyrylcholinesterase by Activity Gel-Scanning Following Non-denaturing Electrophoresis

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Abstract
Butyrylcholinesterase (BChE) in human plasma is composed of four molecular forms: C_1 (monomer); C_2, covalent conjugate between BChE monomer and albumin; C_3, dimer; and C_4, tetramer, the major form. Catalytic parameters of molecular forms were estimated at high substrate concentration after non-denaturing polyacrylamide gel electrophoresis by activity gel-scanning densitometry, using a chromogenic substrate (butyrylthiocholine (BTC)). Though catalytic parameters K_{ss} (dissociation of enzyme substrate complex at high substrate concentration) and catalytic constants are apparent (phenomenological) parameters, results indicate that the four molecular forms of human BChE do not display significant differences in their catalytic behavior at high BTC concentration.

Keywords Butyrylcholinesterase · Molecular forms · Immobilized-enzyme kinetics · Gel scanning

Abbreviations

AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
BTC	Butyrylthiocholine
PAG	Polyacrylamide gel
PAGE	Polyacrylamide gel electrophoresis

1 Introduction

Butyrylcholinesterase (BChE, EC. 3.1.1.8) is catalytically and structurally related to acetylcholinesterase (AChE, EC. 3.1.1.7) [1]. However, unlike AChE, which is involved in terminating the action of the neurotransmitter acetylcholine, no clear function has been established for BChE [2]. However, BChE is an important enzyme involved in metabolism of certain ester-containing drugs and toxicants and in scavenging poisonous carbamyl- and phosphoryl-esters [3].

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BChE is present in skin, organs, and plasma. Its average concentration in plasma is 50 nM. Human plasma BChE is composed of four molecular forms noted C_1, C_2, C_3, and C_4 according to their electrophoretic mobility [4, 5]. C_1, C_3, and C_4 are monomer, dimer, and tetramer; this later is the major form (340 kDa, > 90% total activity in plasma). C_2 is disulfide-bonded BChE monomer to monomeric albumin [6]. Monomer and dimer BChE are thought to be proteolysis products of C_4, produced by clipping off the C-terminal tetramerization domain as for bovine serum AChE [7]. However, there is no conclusive answer for this opinion. These forms can reassociate to make unstable C_4 form [5]. In about 10% of population, a slower band, noted C_5, is present. This band is tetramer bound to a 60 kDa fragment of lamellipodin [8].

BChE does not display Michaelis-Menten behavior with positively charged substrates such as BTC [9–11]. At low substrate concentration, the catalytic behavior is Michaelian-like with corresponding catalytic parameters K_m and $V_{max} = k_{cat}\cdot[E]$. However, at high substrate concentration (> 1 mM), a second substrate molecule binds on peripheral anionic site and forms a ternary complex SES (dissociation constant K_{ss}), and causes substrate activation ($b > 1$): $V''_{max} = b \cdot k_{cat}\cdot[E]$, is the maximum velocity at maximum substrate activation ([S], > K_{ss}). This mechanism can be described by the following Scheme 1 and Eq. 1 in which $< K_m > = 20$ μM, $< k_{cat} > =$ 25,000 min^{-1}, $b \sim 3$, and $< K_{ss} > = 0.5$ mM in 0.1 M phosphate buffer pH = 7.0 at 25 °C.